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# Electron Transfer-oxy Radical Mechanism for Anti-cancer Agents: 9-anilinoacridines

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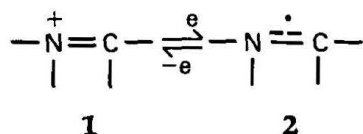
# Electron transfer-oxy radical mechanism for anti-cancer agents: 9-anilinoacridines

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**Summary:** A possible mode of action involving electron transfer is advanced for the 9-anilinoacridines. The mechanism entails formation of toxic oxy radicals which destroy the neoplasm. Cyclic voltammetry was performed on iminium type ions derived by protonation of the acridines. Reductions were generally reversible with potentials of about -0.60 V. Involvement of quinoidal metabolites is also a possibility. The relationship of electrochemical behavior to structure and physiological activity is addressed.

Despite the fact that much insight has been gained in recent years concerning the action of physiologically important drugs, the intimate pathways of operation are generally unknown. A mechanism which is gaining increased acceptance involves redox cycling of the active substance, accompanied by electron transfer (ET). Recent reviews deal with this mode of action (Halliwell & Gutteridge 1985; Sies, 1985; Kappus, 1986). The comprehensive radical mechanism for anti-cancer agents was first proposed more than two decades ago (Holman, 1956; Kovacic, 1959), and has received increasing support, particularly in recent years (Oberley & Buettner, 1979; Kovacic, 1984; Lown, 1985; Kovacic *et al.*, 1986). There are several categories of ET agents, including quinones, metal complexes, aromatic nitro compounds, and iminium moieties. Of the various groups, the iminium class has been the least investigated systematically. Recently the theory was advanced that the iminium ion **1** plays an important role in biological functions (Kovacic, 1984). These species are formed endogenously (Knabe,



1979; Overton *et al.*, 1985). The chief function is thought to be participation in ET: beneficial transformations, interference with normal ET, or generation of toxic oxy radicals. Application of the general theme has been made to carcinogens (Kovacic *et al.*, 1986a) (O-alkyl guanine salt in DNA), antibacterial agents (Ryan *et al.*, 1985; Crawford *et al.*, 1986a; Ames *et al.*, 1986d), CNS agents [benzodiazepines (Crawford *et al.*, 1986b, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (Ames *et al.*, 1986a), phen-cyclidine (Ames *et al.*, 1986b), nicotine (Ames *et al.*, 1986b), spermine (Ames *et al.*, 1986b), anti-malarial agents (Ames *et al.*, 1985), mesoionic compounds (Ames *et al.*, 1986c), anti-cancer drugs (Kovacic *et al.*, 1986b; Crawford *et al.*, 1987a; Crawford *et al.*, 1987b), and amebicides (Ames *et al.*, 1987). The natural phagocytic response to foreign bodies involves attack by activated oxygen (Baehner *et al.*, 1982).

In relation to anti-neoplastic action, the mechanism in some cases presumably

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involves interaction of **1**, usually conjugated, with a substrate, e.g., protein or DNA, entailing electron abstraction. Reduction of **1** should be energetically favorable due to its electrophilic nature (Kovacic, 1984). Oxidation of the radical intermediate **2** by electron donation to an acceptor, e.g., oxygen, resulting in superoxide formation, completes the ET process. Derived oxy species, such as the hydroxyl radical, then exert a lethal effect on the tumor cell by attacking vital cellular constituents. The well known paradox of oncology, that generally the substances which are anti-neoplastic may also induce cancer, gives support to this mechanism of action, since oxy radicals have been implicated in carcinogenesis (Kovacic *et al.*, 1986a,b). The ability to combat this condition may then be linked to the same property. Also many tumor cells are more susceptible than normal ones to elevated concentrations of oxy radicals. A more complete treatment of this subject is presented elsewhere (Kovacic *et al.*, 1986b).

9-Anilinoacridines (Baguley *et al.*, 1981) elicit a wide range of biological responses including anti-tumor effects. The objective of the present study was to determine the electrochemical characteristics of iminium ions derived from these agents. The relationship of electrochemical behavior to structure and physiological activity is addressed. Alternate mechanistic pathways are considered.

## Materials and methods

The eight 9-anilinoacridine compounds were supplied by the Drug Synthesis and Chemistry Branch, Division of Cancer Treatment, National Cancer Institute. The electrolyte used in the electrochemical studies was tetraethylammonium perchlorate (G. F. Smith Chemical Co.); dimethylformamide (DMF, Aldrich Chemical Co.) was obtained in the highest available purity; absolute ethanol (US Industrial Chemical Co.) was used to prepare the aqueous solutions. The appropriate volume of stock solu-

tions of acetic acid and sodium hydroxide was added directly to the electrochemical cell. Acetic acid was used to mimic the weak acids found *in vivo*. Electrochemical solutions were prepared by adding a weighed amount of the test compound and diluting to the desired concentration.

Cyclic voltammetry data were recorded with a PARC model 174A polarographic analyzer connected to a Hewlett Packard model 7035B X-Y recorder. Scan rates generally ranged from 20 to 200 mV s<sup>-1</sup>. All solutions were saturated for 15 min with prepurified nitrogen which was passed through an oxygen scrubbing system. The electrode consisted of a hanging mercury drop (HMDE) working electrode, with a platinum wire as the counter. For each scan a new mercury drop was used. The reference was a saturated calomel electrode (SCE) (Corning). Observed potentials (our work and literature values) were converted to the normal hydrogen electrode (NHE) by addition of 0.24 V to the SCE values. The reported values are the average of two or more measurements involving fresh solutions. The following equations were used for the half-wave potentials and the differences in potentials:

$$E_1 = (E_{pc} + E_{pa})/2$$

$$\Delta E_p = |E_{pa} - E_{pc}| \text{ and}$$

$$E_{pp/2} = |E_{pc} - E_{pc/2}|$$

## Results and discussion

Compound **3a** (Figure 1) as the hydrochloride salt gave an  $E_1$  of  $-0.60$  V in DMF and a  $\Delta E_p$  of 50 mV. The reduction was diffusion controlled as determined from the constant current function (equation (1), below) [data not shown] with an  $i_{pa}/i_{pc}$  value approximately equal to one (Table I). The free base exhibited irreversible reduction at  $-1.26$  V.

$$CF = i_p/V^{1/2} \times C \quad (1)$$

Table I Cyclic voltammetry of substituted acridines<sup>a</sup>

Compound	[HO <sup>-</sup> ]mM	[HOAc]mM	-E <sub>1</sub>	i <sub>pa</sub> /i <sub>pc</sub>	ΔE <sub>p</sub>
3a	—	—	0.60	0.93	50
	—	24	0.61	0.93	50
	0.5	—	1.26 <sup>b</sup>	—	—
	0.5	24	0.78	1.0	70
	0.5	46	0.70	1.0	65
	0.5	66	0.67	0.96	60
3b <sup>c</sup>	—	—	0.60	1.0	50
	0.5	—	1.29 <sup>b</sup>	—	—
	0.5	24	0.74	1.0	60
	0.5	46	0.67	1.0	40
	0.5	66	0.64	0.92	60
3c	—	—	0.59	0.97	60
	0.5	—	1.30 <sup>b</sup>	—	—
	0.5	24	0.74	1.0	65
	0.5	46	0.68	0.94	50
	0.5	66	0.64	0.96	55
3d	—	—	0.60	0.83	55
	1.0	—	1.30 <sup>b</sup>	—	—
	1.0	24	0.73	0.94	70
	1.0	46	0.67	0.91	65
	1.0	66	0.64	0.88	60
3e <sup>c</sup>	—	—	0.59	0.96	65
	0.5	—	1.29 <sup>b</sup>	—	—
	0.5	24	0.73	0.98	60
	0.5	46	0.67	0.84	50
	0.5	66	0.63	0.81	50
3f	—	—	<sup>d</sup>	—	—
	0.5	—	0.61	0.81	90
	1.0	—	1.25 <sup>b</sup>	—	—
	1.0	24	0.71	1.0	90
	1.0	46	0.66	0.82	85
3g	1.0	66	0.61	0.80	75
	—	—	0.40	1.0	50
	0.5	—	0.63 <sup>e</sup> , 1.22 <sup>b</sup>	—	—
	1.0	—	1.22 <sup>b</sup>	—	—
	0.5	24	0.69 <sup>e</sup> , 0.79 <sup>e</sup>	—	—
3h	0.5	46	0.61	1.0	110
	0.5	66	0.57	—	105
	—	—	0.59	0.82	55
	0.5	—	1.30 <sup>b</sup>	—	—
	0.5	24	0.71	0.92	95
	0.5	46	0.67	0.88	80
	0.5	66	0.63	0.88	70

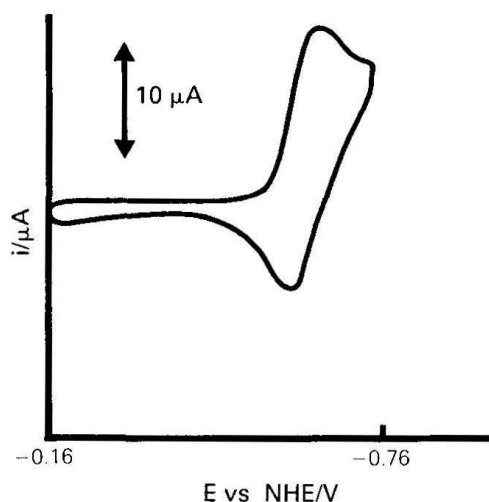
<sup>a</sup> 100 mV s<sup>-1</sup>, tetraethylammonium perchlorate (0.1 M), substrate (0.5 mM, unless otherwise indicated), DMF, HMDE vs NHE, reversible

<sup>b</sup> Irreversible, E<sub>p</sub> value

<sup>c</sup> Substrate, 0.25 mM

<sup>d</sup> Strong adsorption on the electrode, adsorption E<sub>p</sub> about -0.60 V

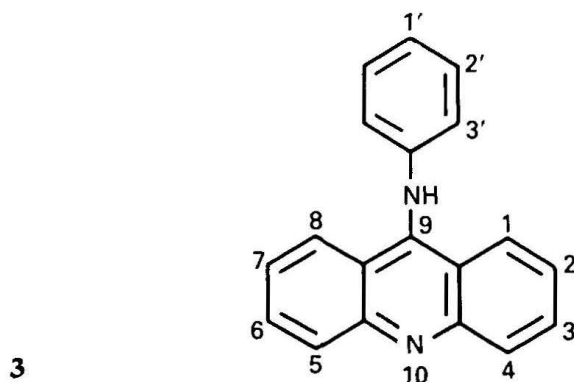
<sup>e</sup> See text for details



**Figure 1** Cyclic voltammogram of **3a** in dimethylformamide, hanging mercury drop electrode, scan rate  $100 \text{ mV s}^{-1}$

Formation of the cation by addition of acetic acid also produced more positive potentials ( $-0.67$  to  $-0.78 \text{ V}$ ) with  $\Delta E_p$  ranging from 60 to 70 mV (Table I), close to the theoretical value of 59 mV for a one-electron process (Bard & Faulkner, 1980).

The ratio of the currents was unity. The remaining acridines exhibited the same type of behavior with the exception of **3f** and **3g**. Compound **3f** (dihydrochloride) adsorbed strongly on the electrode. However, for the monohydrochloride, an  $E_i$  of  $-0.61 \text{ V}$  was observed. The compound behaved similarly to the others under the various remaining conditions. Compound **3g** exhibited an  $E_i$  of  $-0.40 \text{ V}$  as the acid salt. This value is due to the electron-withdrawing effect of the carboxyl group. One equivalent of base produced a quasi-reversible wave ( $\Delta E_p = 120 \text{ mV}$ ) with current of about 40% of the original at an  $E_i$  of  $-0.63 \text{ V}$ . The relatively positive potential is likely due to nuclear protonation by carboxyl. An irreversible peak at  $-1.22 \text{ V}$  was also observed. Addition of 1.0 mM base produced only a wave at  $E_p = -1.22 \text{ V}$  which arises from the unprotonated acridine ring. After addition of acetic acid (24 mM) to the free base, a very broad peak was obtained with an  $E_{pc}$  at  $-0.79 \text{ V}$  and a shoulder,  $E_p \sim -0.69 \text{ V}$ . The broadness may be due to the superposition of two cathodic peaks,



NCS		
Compound number	Substitute	
a	12056	2-OCH <sub>3</sub> ; 6-Cl; 1'-OH·HCl
b	10666	2-OCH <sub>3</sub> ; 6-Cl; 1'-OH; 2'-CH <sub>2</sub> NEt <sub>2</sub> ·2HCl
c	12516	2-OCH <sub>3</sub> ; 6-Cl; 1'-OH; 2'-CH <sub>2</sub> N(CH <sub>2</sub> ) <sub>4</sub> ·HCl
d	17280	2-OCH <sub>3</sub> ; 6-Cl; 1'-OH; 2'-CH <sub>2</sub> N(CH <sub>2</sub> CH <sub>2</sub> Cl) <sub>2</sub> ·2HCl
e	36126	2-OCH <sub>3</sub> ; 6-Cl; 1'-OH; 2'-CH <sub>2</sub> N[(CH <sub>2</sub> ) <sub>7</sub> CH <sub>3</sub> ] <sub>2</sub> ·2HCl
f	32941	3-Cl; 5-CH <sub>3</sub> ; 1'-OH; 2'-CH <sub>2</sub> NEt <sub>2</sub> ·2HCl
g	12509	2-OCH <sub>3</sub> ; 6-Cl; 1'-COOH; 2'-OH·HCl
h	165714	1'-OEt·HCl

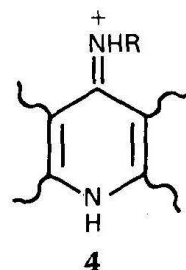
possibly from protonation of the nucleus by both carboxyl and added acid. Stronger concentrations produced a single peak (Table I). The position and nature of the substituents on the acridine ring exert little effect on the reduction potential, cf., **3a** vs **3h** (Table I).

Cyclic voltammetry of **3a** in 50% aqueous ethanol gave  $E_1 = -0.66$  V, with a  $\Delta E_p$  of 40 mV and  $i_{pa}/i_{pc}$  of 0.90 (data not shown). Addition of 0.5 mM base produced a complex wave with  $E_p = -1.06$  V and a shoulder around  $-0.92$  V. Acetic acid (46 mM) resulted in a reversible peak with an  $E_1$  of  $-0.66$  V. Due to the similarity of results to those in DMF, the remaining 9-anilino derivatives were not studied under these conditions.

The effect of the phenyl group is to make reduction more favorable: compare **3a** ( $E_1 = -0.60$  V), mepacrine·H<sup>+</sup> ( $E_1 = -0.72$  V) (Ames *et al.*, 1985), and the parent 9-amino compound ( $E_1 = -0.83$  V) (Breyer *et al.*, 1944).

Related acridines, which display various physiological properties, generally reduce at similar potentials. For protonated amsacrine (*m*-AMSA) [**3**, 1'-NHSO<sub>2</sub>CH<sub>3</sub>; 3'-OCH<sub>3</sub>], reported reduction potentials are  $-0.80$  V (Anderson *et al.*, 1984) and  $-0.74$  V (Crawford *et al.*, 1987a). Other examples are: the antimalarial mepacrine·H<sup>+</sup>,  $E_1 = -0.72$  V (DMF) (Ames *et al.*, 1985); the antibacterial proflavine,  $E_1 = -0.73$  V; and its *N*-methyl salt,  $E_1 = -0.65$  V, both pH 7.3 (Breyer *et al.*, 1944). Anti-neoplastic drugs with the quinolinium nucleus reduced at  $-0.47$  to  $-1.08$  V (Crawford *et al.*, 1987b), and those with fused isoquinolinium structures ranged from  $-0.66$  to  $-1.10$  V (Kovacic *et al.*, 1986b).

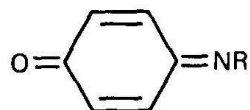
It is important to note that salt formation exerts a favorable influence on both activity and ease of reduction. Various findings indicate a likelihood of aminoacridines functioning in the protonated state (iminium) *in vivo* (Warhurst & Thomas, 1975). Bioactivity is proportional to the percent ionization (Albert, 1985). Intercalation with DNA is thought to be of great significance in the



mechanism of action. Nuclear protonation was associated with maximum activity for *m*-AMSA, which was attributed to facilitation of site binding (Wilson *et al.*, 1981).

The 9-amino group enhances basicity because of cation delocalization. On the other hand, it exerts a pronounced deleterious influence on reduction potential via the same delocalization, illustrated in **4**. Thus, at pH 5.5,  $E_1$  for acridine is  $-0.16$  V and for the 9-amino derivative,  $-0.83$  V (Breyer *et al.*, 1944). An appreciable body of evidence points to steric inhibition of resonance as a consequence of drug binding, which would facilitate ET (Ames *et al.*, 1985). Apparently, binding to DNA entails intercalation of the nucleus with the anilino ring residing in the minor groove (Baguley *et al.*, 1981). X-ray analyses show the side chain at a considerable angle to the acridinium moiety, which appears to be a feature of the molecule rather than crystal-packing requirements.

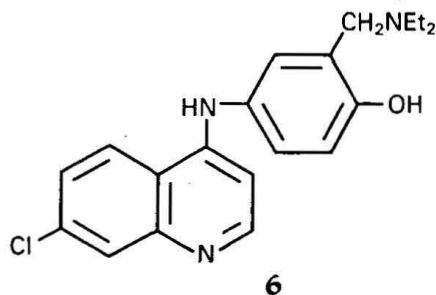
If ET is a mode of action, there may be more than one electroactive site involved for most of our compounds. The *p*-phenylenediamine ring of *m*-AMSA is metabolized *in vitro* by a hepatic microsomal enzyme system producing two oxidation products, diimine and iminoquinone (Shoemaker *et al.*, 1984). Wong *et al.* (1986a) have demonstrated similar oxidation to diimine by Cu(II). The intercalated diimine moiety, complexed with Cu(I), apparently generates oxygen-free radicals in a redox reaction resulting in DNA scission. It is reasonable to suggest that the *p*-aminophenol-type



a, R = H  
b, R = CH<sub>3</sub>CO



drugs in this study (3a–f) may similarly be oxidized to a quinoneimine 5 with analogous production of superoxide. This concept is supported by a number of lines of evidence from related systems. The diimine oxidation product of *m*-AMSA is hydrolyzed to a similar iminoquinone structure (Shoemaker *et al.*, 1984) which exhibits a 100-fold greater toxicity to L1210 cells than *m*-AMSA. The toxicity of *p*-aminophenol (Calder *et al.*, 1979) and acetaminophen (Fischer *et al.*, 1985) are believed to involve derived quinoneimines (5a and 5b, respectively). DNA cleavage has been observed with the related 5-iminodaunorubicin



( $E_1 = -0.46$  V) (Lown *et al.*, 1982). It is conceivable that the anti-malarial agent amodiaquine, 6, operates by such a dual mechanism [iminium and iminoquinone] (Ames *et al.*, 1985).

Possibly, 3d also acts as an alkylating agent similar to the nitrogen mustards. Alkylating species have been postulated as precursors for ET (Kovacic *et al.*, 1986a,b). Both ET sites may then act in concert to increase the potency.

Preliminary findings suggested a relationship between DNA binding and anti-neoplastic ability. However, this factor (intercalation) alone does not explain the differences in activity observed for these agents (Ralph *et al.*, 1983). *m*-AMSA and its derivatives have been shown to induce DNA strand breaks (Ralph *et al.*, 1983), which is commonly associated with generation of oxy radicals (Docampo & Moreno, 1984; Demopoulos *et al.*, 1980). NAD $\cdot$  reduces *m*-AMSA $^+$  to the radical species *m*-AMSA $\cdot$  that is able to convert oxygen to superoxide (Anderson *et al.*, 1984). DNA-

proflavine complexes form hydroxyl radicals upon irradiation (Piette *et al.*, 1982). Structurally related phenazine in quat salt form was observed as an electron mediator in the production of superoxide (Kulkarni & Hodgson, 1980);  $E'_0$  is +0.08 V (Albert, 1968). There have been photo studies demonstrating ET between aminoacridines and DNA (Kittler *et al.*, 1980). Also, ET has been postulated to aid in the binding of aminoacridines to DNA (Atwell *et al.*, 1984). *N*-Methylphenanthridinium salts also undergo ET (Parkanyi & Leu, 1975). It has been proposed that virtually all of the clinical and experimental anti-tumor agents act via disruption of nucleic acid metabolism at some level (Montgomery, 1979). Alternatively, interference with normal ET or with the action of DNA topoisomerase II (Ralph *et al.*, 1983; Wong *et al.*, 1986b) may be involved. *m*-AMSA stimulates the formation of DNA-topoisomerase II complexes which may lead to strand breakage. The cytotoxic effects might be due to interactions with thiol proteins essential for normal cell function (Wong *et al.*, 1986b).

In some cases entailing other anti-neoplastic agents, antimetabolites are clearly involved, e.g. methotrexate (MTX) (Cole, 1970) and  $\alpha$ -difluoromethylornithine (DMFO) (Metcalf *et al.*, 1978). There are recent indications that entities are generated as a result of enzyme binding, which display the potential for ET (Kovacic *et al.*, 1986b). For example, with MTX, iminium is formed as a result of protonation by an aspartic acid residue from dihydrofolate reductase (Howell *et al.*, 1986). The favorable reduction potential ( $E_1 = -0.044$ – $-0.0675$  pH, aqueous buffer) (Gurira & Bowers, 1983) of the resulting cation creates the possibility of participation in electrophysiological reactions. Similarly DFMO is known to combine with the pyridoxal moiety of ornithine decarboxylase (Metcalf *et al.*, 1978). In the process, conjugated imine is generated which can act as a precursor of iminium or a metal complex. Again, ET appears plausible. A recent fascinating finding involves application of the same principle to the  $\beta$ -lactam area (Kovacic

*et al.*, 1987). The well-documented inhibition of bacterial cell wall enzyme is generally thought to be the sole event responsible for activity. It is remarkable that lactam ring opening can lead to an imine forerunner of conjugated iminium. Model compounds containing these functionalities (iminium with attached carboxyl) demonstrated quite favorable reduction potentials ( $-0.39$  to  $-0.61$ , pH 3.7 to 5.3). ET could result in lethal disruption of normal electron transport chains.

#### Other considerations

According to the theory, the different agents act *in vivo* as ET entities. Reports indicate that reduction potential *in vivo* may well be better than *in vitro* (Barton *et al.*, 1986; Ames *et al.*, 1986c). These aspects are treated in more detail elsewhere (Ames *et al.*, 1986b; Crawford *et al.*, 1986a).

Is it reasonable to expect that the electrochemical characteristics of the drugs could translate into physiological activity? Prior reports indicate such a relationship; e.g., Hodnett *et al.* (1978) were able to identify the anti-tumor activity of 75% of the investigated iminobenzoquinones correctly based only on their reduction potentials. Antibacterial mitomycins possessing less negative  $E_1$  values exhibit more powerful activity (Kinoshita *et al.*, 1971). A study of 9-methylaminoacridines revealed a symbiotic relationship between electrochemical properties and antibacterial activity (Shapovalov *et al.*, 1980). Correlations have been drawn between toxicity and reduction potential for a number of aromatic and heterocyclic nitro compounds (Chignell, 1985), including radiosensitizers.

The present theory is clearly an oversimplification of a very complex situation. There is important involvement of other factors, primarily inhibition of DNA synthesis, DNA defect repair, antimetabolite action, and immunological responses (Kovacic *et al.*, 1986b). An example of a drug that is not readily accommodated by our approach is 5-fluorouracil (Will & Dolnick, 1986). It is

conceivable that several mechanisms operate in concert for certain drugs.

To summarize, based on the evidence from our work and prior contributions, the various agents appear to fit the working hypothesis: binding (intercalation) to DNA, involvement in ET, and production of activated oxygen. However, in certain cases there are gaps that require filling.

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